

IAP20 Rec'd PCT/PTO 10 FEB 2006

Amendments and Written Reply for International Application No. PCT/RO2004/000012
(under Article 19)

The amendments decided by the applicant as result Written Opinion of the International Searching Authority concern the inventive step.

The applicant, Institutul Oncologic "Prof. Dr. Al. Trestioreanu" Bucuresti, specify that the respective three concentrations indicated in claim one 1 weren't the only concentration for which the method disclosed by the invention has been used.

The respective three concentrations were only the concentration chosen by the applicant to by exemplified in the description.

The applicant has been using for many years for many concentrations of Deuterium Depleted Water (from 5 ppm to 120 ppm) in order to objectively determine the effects of the tumoral regression, the inhibiting effects over the development of the tumors Walker 256 and T8 Guérin were for many of these concentrations statistically insignificant for the method of exploitation in that the applicant wished to determine the most efficient concentration. During the searches performed by the applicant using the multitude of concentrations, there has been improved and completed the method so that it is more objective, reproducible and exact. Globally, the method, (with all its claimed phases), presents inventive step from the document D3(US 5.855.921 A) where it isn't retrieved and qualified persons couldn't realize it on the base of the data of this document US 5,855.921 A.

So the respective three concentrations indicated in the international application:

- are not limitative for using the method according the invention
- are not arbitrary chosen by the applicant
- their enumeration was made by the applicant for the invention exemplification

The applicant can prove this aspect by documents and data, at the examiner request.

Given the written opinion of the International Searching Authority concerning the invention understanding, the applicant redrafted the claims and page 3 from the description, documents herewith enclosed.

In the followings the applicant wish to reply to some opinions of the examiner concerning the inventive step of the method – object of international application.

- I. The applicant wish to precise that no elements presented by the examiner at items b) and c) from Written Opinion couldn't be considered from biological and experimental point of view as being equivalent with those of the document US 5,855.921 A. It is known the difference the test animals outbred (used in invention) and the inbred animals(used by US 5,855.921 A) as regards their reply to the tumor graft, because there is permitted a more easily extrapolation of the results on the human being than that ones obtained on singeneic animals. The applicant hasn't used test animals outbred only as an equivalent alternative to the inbred animals, but the applicant knew that these animals used by US 5,855.921A are strongly immunosuppressed and this aspect could induce nonsense results in determination of the efficient concentration.
An important differences between the administrating way of the Deuterium Depleted Water at the test animals is the fact that the method according to the invention provide the administering of Deuterium Depleted Water before tumor graft, unlike the US 5,855.921 A where such an administering isn't provided.

Another essential difference between the applicant's method and US 5,855.921 A is the way in that the tumoral transplant is achieved. The claimed method provide that the tumoral transplant be subcutaneously done on HEALTHY ANIMALS, unlike US 5,855.921 provide a xenotransplant on animals strongly immunologic suppressed, fact that from the searches performed by the applicant, it is proved that it induce nonsense results concerning the anti-tumor efficiency. In US 5,855.921 A, the treatment starts after the tumor graft after a significant period of 24 days. The treatment from US 5,855.921 starts with a certain dose, than after a relatively short period, the treatment continues with another dose without being obvious in the patent description, the reason of this treatment schema. Also, the treatment schema wasn't in the international application and its applicant considers from this reason that the invention cannot be obvious in regard to this document.

The applicant is ready to provide all the necessary information to the examiner that will do the substantive examination.

- II. The steps mentioned at item d) and e) in Written Opinion are considered by the applicant very important, they being part of the ensemble of the claimed method. They have resulted during the searches as being necessary and as results they are part of the method conception. The explanation are as follows:
- determination of the animals weight during the test is a very important check point for the evaluation of the biological status of the animal, and even this determination looks like being standard, in the ensemble of the invention method this step is compulsory, the animal's weight proving its general physiological status during the experiment, and the weight maintenance is an objective check point proving that there aren't changes in the general health status of the animal.
 - the tests indicated at item e) from the Written Opinion are also absolutely necessary because they accentuates the immunological status of the animals treated with Deuterium Depleted Water; they objectively shows whether this water administering has humoral and cellular immunological stimulated or not; as results, this step is also one compulsorily belonged to the method in its ensemble and cannot be appreciated besides these one as being claimed alone.
 - determination of the cells viability going to be grafted is also a very important step of the method, due to the fact the level of the viability of the cancer cells going to be grafted (living cells number/dead cells number) has to be 98%, so that the by the tumor graft is obtained a cancer development in proportion of circa 100% and as results the most efficient concentration result is the most objective result. In US 5,855.921 A the viability test of the cancer cells isn't remembered, that means it is neither used, fact that is a great disadvantage of this invention of this US patent due to the fact that the less of this test assume the existence of some error possibility in determination of the efficient concentration of Deuterium Depleted Water.
 - in the method according the invention isn't claimed the use of the blue tripan but the blue tripan is claimed in the step of the viability of the cancer cells going to be grafted.

It is obvious that the method steps are chosen for create an objective character to the method according the invention and they aren't retrieved in the state of art so that together being part of this method and it is neither based on the on trial and error method as examiner thinks.

The ensemble of the steps is the method according the invention and they cannot be evaluated as separate inventive level, one by one.

The applicant doesn't claim the way of performing of the test and he neither alleged that their performing would be done otherwise than in the known conditions. Applicant has claimed an unitary ensemble of steps that have to be taken in order that the method achievement be objective.

- III. Method according the invention that provide the administering the Deuterium Depleted Water and the prophylaxis of the cancer tumor, that in the presented state of art this aspect is not retrieved. Moreover, in the method according the invention it is provide that the once started administering the Deuterium Depleted Water has to be continuously, any ceasing of the treatment determining the decrease of the inhibition of the malign tumor development. Neither this aspect is retrieved in the presented state of art and the treatment of US 5,855.921 A is strictly limited to 90 days.

These new elements of the method have resulted after years of searches within Institutul Oncologic Bucuresti and they contain sure inventive step.

- IV. As regards claim 2 of the international application, the applicant specifies that from all experimented concentrations within 5 ppm to 120 ppm the most significant ones were three concentrations of Deuterium in the depleted water namely 25ppm, 60ppm si 100 ppm, concentrations that had significant anti-tumor effects. From these three concentrations, that of 60 ppmD is proved as having the most efficient anti-tumor effects.

The authors of the method of US 5,855.921 A have used Deuterium Depleted in concentration of 0,1ppmD – 110ppmD, but in the description there is ascertain no concentration that to be distinguished by an optimal efficiency. **Also from US 5,855.921 A there doesn't results that in the ascertainment of an efficient concentration was a technical problem for the achievement of the invention.**

It could be shown that between these two invention, the application of Instiutului Oncologic Bucuresti and US 5,855.921, the technical problems that are going to be solved are different and as results there has to be taken into account this aspect too at the evaluation of the inventive activity.

- V. Method accordingly the invention differs of the others two documents of the state of art and claimed by the Written Opinion:
- D1: Deuterium Depleted Water in concentration of 99ppmD was added to the growth medium in order to evaluate its effect over the rate of growth of the mice's normal-fibroblast cells and over mice's A4 tumor cells. This fact proves that the technical problem from this document isn't the same with that one from the invention and the method could be either considered as being obvious in this document.
 - D2: is referring to administering a Deuterium Depleted water with a concentration of 115ppD at 18 days from the tumor transplant with the administering period of 16 days or accordingly to another experiment of this document the concentration was of 30ppmD, administering was done at 32 days since the tumor graft, but during the experiment the concentration was risen to 110-1120 ppmD, without the necessary medical explanation. As results, the method of the international application cannot be considered as being obvious of this document

Technical issue the invention is solving is the establishing of a method for experimental determination in vivo of an efficient Deuterium content in water, in order to obtain optimum results in cancer therapy on rats.

According to the invention, the method consist in Deuterium Depleted Water administering before and after tumor grafting on animals, following the stages below:

- A) Deuterium Depleted Water administering to Wistar outbred rats by diet, with concentration less than 100 ppm, over a period of 60 days, simultaneously to dieting a control group of animals with water having 150 ppm content of Deuterium (tap water), over the same period of time
- B) Viability determination for the tumor cells to be grafted, using tripan blue
- C) Grafting of the animals in the experimental group and the control animals in the 60th day, subcutaneous, with 1×10^7 malign tumor cells in 0,5 ml normal saline solution of 256 Walker sarcoma (the solid tumor) and T8 Guérin lymphotropic epitelioma (solid tumor), both of them having cells with a viability over 98%.
- D) Continuously and long-term administering, by diet, of Deuterium Depleted Water, with concentration less than 100 ppm deuterium, period over which the followings are to be done:
 - a) Starting with the 4-th post-graft day the tumor nodules measurement and examination is performed on each 2-3 days;
 - b) Monitoring of animals physiological condition by weekly weighing, monitoring their food and water consumption, notifying the toxic condition occurrence
 - c) After 60 days, when all the animals in control group are dead, preferable between the 160th and 200th day after graft, the effect produced by administering of established concentration of Deuterium Depleted Water is observe don the surviving animals homeostasis from experimental groups, respectively the way how humoral immune system and cellular immune system of these animals has been influenced, by performing of a series of examination on immunological condition of the animals, namely: leucocytes formula test to establish lymphocytes and blastic cells levels; hematopoietic marrow tests to establish the plasmocytes and NK-K cells levels.
- E) Determination of efficient concentration of Deuterium Depleted Water for tested surviving animals depending on new homeostasis occurrence, and on the results obtained related to tumoral regression, as well as to cancer curing.

AMENDED CLAIMS

1. The method for in vivo determination on tested animals of the efficient concentration of Deuterium Depleted Water for cancer therapy is **characterized by the fact that** it provides Deuterium Depleted Water administering to tested animals before and after tumor grafting with animal grafts and it takes the following steps:

- A) Deuterium Depleted Water administering to Wistar outbred rats by diet, with concentration less than 100 ppm, over a period of 60 days, simultaneously to dieting a control group of animals with water having 150 ppm content of Deuterium (tap water), over the same period of time..
- B) Viability determination for the tumor cells to be grafted, using tripan blue
- C) Grafting of the animals in the experimental group and the control animals in the 60th day, subcutaneous, with 1×10^7 malign tumor cells in 0,5 ml normal saline solution of 256 Walker sarcoma (the solid tumor) and T8 Guérin lymphotropic epithelioma (solid tumor), both of them having cells with a viability over 98%.
- D) Continuously and long-term administering, by diet, of Deuterium Depleted Water, with concentration less than 100 ppm deuterium, period over which the followings are to be done:
 - a. Starting with the 4-th post-graft day the tumor nodules measurement and examination is performed on each 2-3 days;
 - b. Monitoring of animals physiological condition by weekly weighing, monitoring their food and water consumption, notifying the toxic condition occurrence
 - c. After 60 days, when all the animals in control group are dead, preferable between the 160th and 200th day after graft, the effect produced by administering of established concentration of Deuterium Depleted Water is observe don the surviving animals homeostasis from experimental groups, respectively the way how humoral immune system and cellular immune system of these animals has been influenced, by performing of a series of examination on immunological condition of the animals, namely: leucocytes formula test to establish lymphocytes and blastic cells levels; hematopoietic marrow tests to establish the plasmocytes and NK-K cells levels.
- E) Determination of efficient concentration of Deuterium Depleted Water for tested surviving animals depending on new homeostasis occurrence, and on the results obtained related to tumoral regression, as well as to cancer curing.

2. Method, as per claim no. 1, **characterized by the fact** that it determines the 60 ppm Deuterium Depleted Water as the concentration that is the most efficient for cancer therapy and prophylaxis by continuously and long-term administering of this type of water as a daily diet.